Long Term Effects of Acid Rain on an Alpine Plant Population II: Recovery of *Aquilegia caerulea* Following Exposure to Simulated Acid Rain

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Long term effects of acid rain on an alpine plant population II: Recovery of *Aquilegia caerulea* following exposure to simulated acid rain

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INTRODUCTION

Despite intensive research on acid rain effects in terrestrial and aquatic communities, little is known about long term effects of acid rain on vegetative and reproductive processes in alpine plant communities. Funke and Bonde (1986) investigated short term acid rain effects on reproductive processes in *Acomastylis rossii* and *Bistorta vivipara* and concluded that flower production was reduced in *Acomastylis* but enhanced in *Bistorta*. Interpretation of short term studies is made difficult by the possibility that a response observed in a single season may be achieved at the expense of future seasons, as plants adjust energy allocation to vegetative and reproductive growth. In addition, the response observed in a single season cannot reflect longer term developmental responses that may result from exposure to acid rain stress.

In 1989 we initiated a long term experiment to study the effects of acid rain on vegetative and reproductive processes in *Aquilegia caerulea* L. (Ranunculaceae) at the Glacier Lakes Ecosystem Experiments Site in the Snowy Mountains of southwest Wyoming. *A. caerulea* is a rhizomatous perennial herb common in the central Rocky Mountains at elevations from 2,000-3,600 meters. Our study site is adjacent to Meadow Creek at an elevation of approximately 3340 meters. This population usually flowers between early July and late August, depending on the date of snow melt in the alpine region. Hand pollination experiments have demonstrated that *Aquilegia caerulea* produces fewer seeds and lighter seeds after exposure to simulated acid rain (McKenna, 1990, 1992). *A. caerulea* pollen germination in vitro is very sensitive to low pH, decreasing to less than 3% germination at pH 3.5 (McKenna, 1990, 1992). Average pollen tube length in vitro also decreases in media with pH below 4.5.

In 1990 we divided newly emerging *Aquilegia caerulea* plants into 3 groups for a long-term study of acid rain effects on vegetative growth. One group was exposed throughout the growing season to repeated sprays of acid rain simulant (pH 3.5), one group was exposed to ambient rain simulant (pH 5.6) and the third group was maintained as a non-sprayed control. Plant growth was monitored throughout the growing season. Plants in the acid rain treatment group had significantly shorter stem heights than plants in the ambient rain or non-spray treatments (McKenna, 1992). The experiment was continued through 1993 and the *Aquilegia* plants exposed to acid rain simulant continued to show reduction in vegetative growth compared to plants exposed to the ambient rain simulant (McKenna, 1993, 1994).

This paper reports on the final stage of this study. This stage was designed to examine the

extent of recovery or long term damage in this population after exposure to the acid stress was halted. Nearly all alpine plants are perennials, so long term effects will be particularly important in this ecosystem. Since the process of establishment of seedlings is very difficult in alpine plant communities (Chambers, 1995), damage to adult plants can lead to widespread community effects. We wished to find out whether the decreased vegetative growth exhibited by *Aquilegia* plants in the acid spray treatment would persist during two growing seasons after the treatments were stopped.

METHODS

In June 1994, we relocated individual *Aquilegia* plants from the Meadow Creek population that were tagged in 1990. We measured each plant weekly over a 9 week period from June 30- September 1. We measured plant height, crown diameter, and the height of 5 marked central stems on each plant. We also counted the number of stems, the number of buds, the number of flowers, and the number of fruits on each plant. We assessed plant vigor by qualitatively determining the percent leaf area that was chlorotic or mottled. At the end of the 10 week monitoring period, we marked the location of each plant with a pin flag to aid in locating them the following year.

In Summer 1995, the snow cover at Meadow Creek lasted until early August, so plant emergence was much later than previous years. We relocated the plants as they emerged in early August and measured them four times over a 5 week period from August 7-September 15th. We made the same measurements in 1995 as we did in 1994, but we did not include a measurement of the height of 5 central stems per plant or the total number of stems per plant.

RESULTS

We relocated all 15 plants in the acid-spray group in 1994 and all of them survived until the eighth week (8/25) when 3 plants senesced. We found all 15 plants in the ambient-spray group in 1994; one plant died during the second week (7/28) and the remaining 14 plants survived through the eighth week. We relocated 14 of the 15 plants in the non-spray group in 1994 and all 14 survived through the eighth week. There was rapid mortality of above ground plant parts in all three treatment groups during the ninth week. At the ninth measurement date there were only 4 remaining plants in the acid-spray group, 6 remaining plants in the ambient spray group and 4 remaining plants in the non-spray group, so we concluded the study at this point. The 1994 study was the most comprehensive study we completed, since we were able to follow the majority of plants from emergence to senescence.

The mean height of plants from the three treatment groups at all measurement dates is shown in Table 1. We chose four measurement dates for statistical analysis: Date 2 = 7/6, Date 4 = 7/21, Date 6 = 8/4 and Date 8 = 8/17. We carried out an analysis of variance (ANOVA, Systat

5.04) on log-transformed values for plant height on each date, and the results are shown in Table 2. There was a significant effect of previous spray treatment on plant height at all measurement dates (Figure 1). We performed a pairwise comparisons test (Tukey test, Systat 5.04) following the anova for each measurement date. Plants from the acid spray group were significantly shorter than plants from the ambient spray group on dates 2, 6 and 8. Plants from the acid spray group were significantly shorter than plants from the non-spray group on all measurement dates. There was no significant difference between the height of plants from the ambient-spray and non-spray groups on any of the measurement dates.

We also performed an analysis of variance on log-transformed values for crown diameter of plants in each treatment group and on the number of stems per plant in each treatment group. There was no significant effect of previous spray treatment on either of these variables. There were no significant effects of previous spray treatment on the mean number of buds/plant, mean number of flowers/plant or mean number of fruits/plant, but there was great interplant variation in these reproductive characters. The plants in the acid spray group produced much fewer total buds, flowers and fruits than plants in either the ambient spray group or the nonspray group (Table 3).

Our final analysis of the data obtained in 1994 was a nested analysis of variance (MGLH, Systat 5.04) performed to examine the effects of previous spray treatment on the height of 5 central stems from plants in each group (Table 4). On all four dates, the effect of treatment on stem height was highly significant ($p<.0001$) and the individual plant contribution to variation was highly significant ($p<.0001$). The stem heights from plants in the acid-spray group were significantly shorter than plants in the ambient-spray group in the later part of the study (dates 6 and 8). The stem heights from plants in the acid-spray group were significantly shorter then plants in the non-spray group on all four dates. The stem heights of plants in the ambient spray treatment were significantly shorter than plants in the non-spray group on the first 3 measurement dates, but there was no significant difference in stem height between these two treatments by the end of the study. Mortality of the marked central stems was very low (< 5%) in all treatments for the first four weeks of the study , but it increased to 16% during week 6 and 33-44% during the eighth week.

In Summer 1995, we located all 15 plants in the acid-spray group and all plants in this group survived through the last measurement date. We located 14 of the 15 plants in the ambient spray and nonspray group and all 14 plants in each group survived through the last measurement date. This was the latest growing season we observed since the beginning of the study in 1989, since the snowpack remained at the study site until early August.

We carried out an analysis of variance (ANOVA, Systat 5.04) on log-transformed values for plant height and crown diameter on each date, and only the results for plant height showed significant treatment effects (Table 5). On Date 2 and Date 3 the effects of previous spray treatment had a significant impact on plant height (Date 2: $p = 0.04$; Date 3: $p = 0.006$) , but by the last measurement date these effects were no longer significant ($p = 0.09$). We performed a

pairwise comparisons test (Tukey test, Systat 5.04) following the anova for each measurement date. Plants from the acid spray group were significantly shorter than plants from the ambient spray group on date 3 (Figure 2). Plants from the acid spray group were significantly shorter than plants from the non-spray group on date 2 and 3. There was no significant difference between the height of plants from the ambient-spray and non-spray groups on any of the measurement dates.

We carried out an analysis of variance (ANOVA, Systat 5.04) on the mean number of buds/plant, mean number of flowers/plant or mean number of fruits/plant for each treatment group at each measurement date and only results for the number of buds showed significant treatment effects (Table 6). On Date 1 and Date 4 the effects of previous spray treatment had a significant impact on the number of buds produced per plant (Date 1: $p = 0.04$; Date 4: $p = 0.02$). We performed a pairwise comparisons test (Tukey test, Systat 5.04) following the anova for each measurement date. Plants from the acid spray group produced significantly fewer buds per individual than plants from the ambient spray group at the beginning of the growing season (Date 1). Plants from the acid spray group and the ambient spray group produced significantly fewer buds per individual than plants from the non-spray group at the end of the growing season (Date 4). In 1995 as in the previous year, the plants from the acid spray group produced far fewer total number of buds, flowers and fruits than plants in the ambient spray or nonspray groups (Table 7).

DISCUSSION

The effects of exposure to simulated acid rain in *Aquilegia caerulea* clearly can persist for more than one growing season, even when acid rain stress is no longer present. Despite cessation of the spray treatments in 1994, the plants from the previous acid spray group were significantly shorter than plants from the previous ambient spray and nonspray groups. The nested anova for effects of previous spray treatment on stem height indicate that although genetic and microenvironmental factors influencing individual plants have a significant effect on stem height, significant effects due to previous spray treatment history still persist. In 1995, the plant height differences were seen again, although a wider variation between plants in the treatments resulted in significant differences between treatments during the middle two measurement dates only. This increase in the variation between plants in each group suggests that the lack of ongoing spray treatment may allow other genetic and environmental differences between plants to exert a stronger effect.

Effects of acid rain exposure on reproductive output expressed as reductions in the total production of buds, flowers and fruits was seen in both 1994 and 1995. There is high interplant variation in these reproductive characters which may reflect differences in plant genetic makeup as well as differences in tolerance to the acid rain stress. Reductions in vegetative growth of plants are frequently accompanied by reductions in flower production primarily due to energy limitations, but some species respond to environmental stress by increasing reproductive effort (Levitt, 1989). We can estimate from our data that approximately 37-55% of buds initially

develop into fruit, but we have no firm data on the proportion of fruits that produce viable seeds. Previous studies in this population have demonstrated reduced seed production and reduced seed weight in plants exposed to acid rain prior to hand pollination (McKenna, 1992). The combined effects of decreased flower production and decreased seed production could seriously impact the reproductive potential of *A. caerulea* plants exposed to acid rain stress.

The plants in all three treatment groups were larger in 1995 than in 1994; this may reflect a greater availability of water due to the lateness of snowmelt in 1995. The increased growth in the acid spray group may also indicate some potential for recovery in this group since the height of plants 4 weeks after emergence (Date 3) is higher than seen in any previous year at an equivalent stage in the growing season. The resilience of this species is also impressive, since the plants in the acid-spray group suffered no mortality during the four years of spray treatments or the two years following that period, even though there were clear growth reductions resulting from exposure to the acid rain stress. A similar stress applied to these plants in the seedling stage might have a stronger effect on mortality.

In previous studies with *Aquilegia caerulea* (McKenna, 1990, 1992, 1993), we have demonstrated that *Aquilegia* pollen exhibits reduced germination and growth in a low pH environment (below pH 4.5). The overlap in sensitivity of gametophytic tissues (pollen) and sporophytic tissues in this species suggests that this sensitivity may reflect a basic metabolic system that is intolerant to low pH. This overlap in sensitivity also suggests that pollen germination tests may be useful in screening plant species to determine their relative sensitivity to acid rain stress. The results of this investigation of acid rain effects on vegetative and reproductive processes in *Aquilegia caerulea* suggests that acid rain can reduce growth and reproduction in this species, and that these changes may persist even after the stress factor has been removed.

LITERATURE CITED

Chambers, J.C. 1995. Relationships between seed fates and seedling establishment in an alpine ecosystem. Ecology 76: 2124-2133.

Funk, D.W. and E.K. Bonde. 1986. Effects of artificial acid mist on growth and reproduction of two alpine plant species in the field. lAmer. J. Bot. 73:524-528.,

Levitt, J. 1989. *Response of Plants to Environmental Stress*. Blackwell, London.

McKenna, M. A. 1990. Atmospheric effects on plant reproduction. Final report to the U.S. Forest Service, Rocky Mountain Forest and Range Experiment Station, Fort Collins, Co.

McKenna, M. A. 1992. Genotypic and phenotypic components of alpine plant response to acid rain. Final report to the U.S. Forest Service, Rocky Mountain Forest and Range Experiment Station, Fort Collins, Co.

McKenna, M. A. 1993. Mechanisms of alpine plant response to acid rain. Final report to the U.S. Forest Service, Rocky Mountain Forest and Range Experiment Station, Fort Collins, Co.

TABLE 1.

AQUILEGIA CAERULEA 1994

MEAN PLANT HEIGHT IN THREE SPRAY TREATMENT GROUPS

DATE	ACID-SPRAY	AMBIENT- SPRAY	NON-SPRAY
6-30-94	Y = 8.71 s = 3.09 n = 15	Y = 12.13 s = 5.06 n = 15	Y = 12.55 s = 5.23 n = 14
7/6/94	Y = 10.89 s = 2.54 n = 15	Y = 16.38 s = 5.79 n = 14	Y = 15.16 s = 4.70 n = 14
7/14/94	Y = 11.25 s = 4.19 n = 15	Y = 16.62 s = 7.80 n = 14	Y = 16.06 s = 5.09 n = 14
7/21/94	Y = 12.69 s = 3.51 n = 15	Y = 17.46 s = 6.62 n = 14	Y = 17.77 s = 4.94 n = 14
7/28/94	Y = 13.05 s = 3.64 n = 15	Y = 18.32 s = 5.53 n = 14	Y = 18.38 s = 3.10 n = 14
8/4/94	Y = 13.44 s = 4.54 n = 15	Y = 18.57 s = 5.92 n = 14	Y = 19.10 s = 4.42 n = 14
8/10/94	Y = 14.07 s = 4.20 n = 15	Y = 18.28 s = 5.39 n = 14	Y = 18.43 s = 3.66 n = 14
8/17/94	Y = 13.88 s = 4.60 n = 15	Y = 18.04 s = 5.32 n = 14	Y = 18.40 s = 4.74 n = 14
8/25/94	Y = 13.92 s = 3.73 n = 12	Y = 17.61 s = 4.95 n = 14	Y = 17.93 s = 4.68 n = 14

TABLE 2. ANALYSIS OF VARIANCE FOR PLANT HEIGHT AQUILEGIA 1994.

ATE = 2.000

DEP VAR: PLTHT N: 44 MULTIPLE R: 0.520 SQUARED MULTIPLE R: 0.2

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
TRTMNT	282.737	2	141.368	7.598	0.002
ERROR	762.891	41	18.607		

)ATE = 4.000

DEP VAR: PLTHT N: 43 MULTIPLE R: 0.485 SQUARED MULTIPLE R: 0.2

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
TRTMNT	290.706	2	145.353	6.135	0.005
ERROR	947.655	40	23.691		

DATE = 6.000

DEP VAR: PLTHT N: 43 MULTIPLE R: 0.481 SQUARED MULTIPLE R: 0.2

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
TRTMNT	304.886	2	152.443	6.025	0.005
ERROR	1012.022	40	25.301		

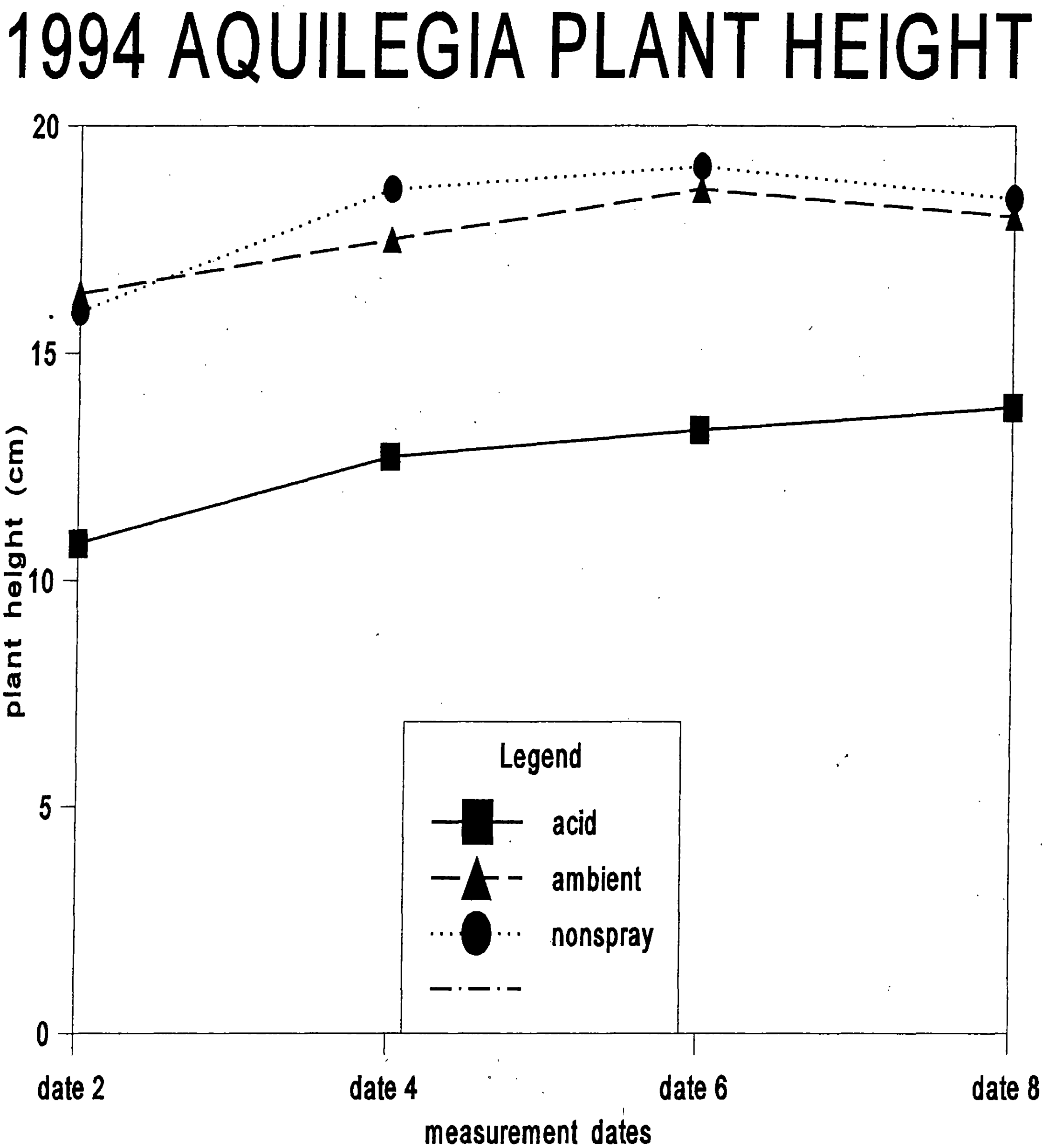
DATE = 8.000

DEP VAR: PLTHT N: 43 MULTIPLE R: 0.405 SQUARED MULTIPLE R: 0.1

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
TRTMNT	191.910	2	95.955	3.932	0.028
[illegible]	[illegible]	[illegible]	[illegible]		

FIGURE 1.



1994 AQUILEGIA PLANT HEIGHT
20
15
10
5
0
plant height (cm)
Legend
acid
ambient
nonspray
date 2
date 4
date 6
date 8
measurement dates

TABLE 3.

Aquilegia caerulea
1994

TOTAL NUMBER OF BUDS, FLOWERS AND FRUITS									
	Acid			Ambient			Non-Spray		
Date	Buds	Flower	Fruit	Buds	Flower	Fruit	Buds	Flower	Fruit
7-6	67	0	0	136	4	0	143	2	0
7-21	100	11	8	196	42	16	115	28	8
8-4	35	24	19	11	19	81	16	28	28
8-17	0	1	54	0	5	93	0	0	67
TOTALS	202	36	81	343	70	190	274	58	103

TABLE 4. NESTED ANALYSIS OF VARIANCE FOR STEM HEIGHT AQUILEGIA 1994.

DATE = 1.000

DEP VAR: STEMHT N: 223 MULTIPLE R: 0.895 SQUARED MULTIPLE R: 0.8

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
TRTMNT	3.414	2	1.707	31.986	0.000
PLTNO {TRTMNT}	35.067	42	0.835	15.645	0.000
ERROR	9.500	178	0.053		

DATE = 2.000

DEP VAR: STEMHT N: 217 MULTIPLE R: 0.907 SQUARED MULTIPLE R: 0.8

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
TRTMNT	1.432	2	0.716	16.541	0.000
PLANTNO {TRTMNT}	33.363	42	0.794	18.350	0.000
ERROR	7.446	172	0.043		

DATE = 3.000

DEP VAR: STEMHT N: 190 MULTIPLE R: 0.905 SQUARED MULTIPLE R: 0.8

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
TRTMNT	5.298	2	2.649	72.664	0.000
PLANTNO {TRTMNT}	17.705	41	0.432	11.845	0.000
ERROR	5.323	146	0.036		

DATE = 4.000

DEP VAR: STEMHT N: 141 MULTIPLE R: 0.926 SQUARED MULTIPLE R: 0.8

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
TRTMNT	2.896	2	1.448	51.869	0.000
PLANTNO {TRTMNT}	12.629	40	0.316	11.309	0.000
ERROR	2.736	98	0.028		

TABLE 5. ANALYSIS OF VARIANCE FOR PLANT HEIGHT AQUILEGIA 1995.

DATE = 1.000

DEP VAR: PLTHT N: 43 MULTIPLE R: 0.279 SQUARED MULTIPLE R: 0.0

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
TRTMNT	0.675	2	0.337	1.683	0.199
ERROR	8.014	40	0.200		

DATE = 2.000

DEP VAR: PLTHT N: 43 MULTIPLE R: 0.386 SQUARED MULTIPLE R: 0.1

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
TRTMNT	2.301	2	1.150	3.503	0.040
ERROR	13.135	40	0.328		

DATE = 3.000

DEP VAR: PLTHT N: 43 MULTIPLE R: 0.475 SQUARED MULTIPLE R: 0.2

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
TRTMNT	3.123	2	1.561	5.816	0.006
ERROR	10.739	40	0.268		

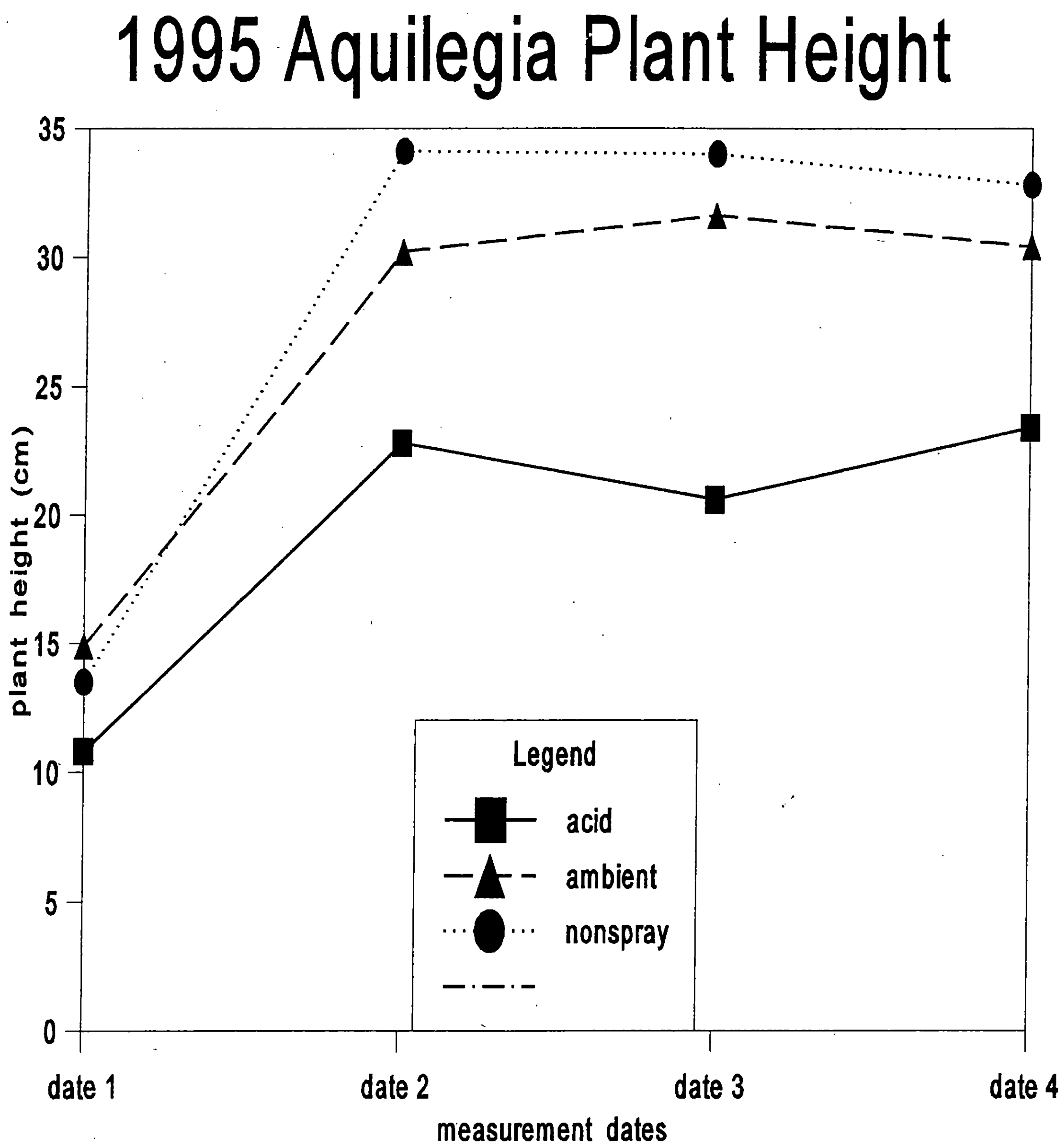
DATE = 4.000

DEP VAR: PLTHT N: 43 MULTIPLE R: 0.339 SQUARED MULTIPLE R: 0.1

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
TRTMNT	1.681	2	0.840	2.598	0.087
ERROR	12.940	40	0.324		

FIGURE 2.



1995 Aquilegia Plant Height
35
30
25
20
15
10
5
0
plant height (cm)
Legend
acid
ambient
nonspray
date 1
date 2
date 3
date 4
measurement dates

TABLE 6. ANALYSIS OF VARIANCE FOR # BUDS/PLANT AQUILEGIA 1995.

DATE = 1.000

DEP VAR: NOBUD N: 43 MULTIPLE R: 0.386 SQUARED MULTIPLE R: 0.1

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
TRTMNT	51.604	2	25.802	3.501	0.040
ERROR	294.814	40	7.370		

DATE = 4.000

DEP VAR: NOBUD N: 43 MULTIPLE R: 0.417 SQUARED MULTIPLE R: 0.1

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
TRTMNT	3.932	2	1.966	4.201	0.022
ERROR	18.719	40	0.468		

TABLE 7.

Aquilegia caerulea
1995

	TOTAL NUMBER OF BUDS, FLOWERS AND FRUITS								
	Acid			Ambient			Non-Spray		
Date	Buds	Flower	Fruits	Buds	Flower	Fruits	Buds	Flower	Fruits
8-7	6	1	0	40	2	0	35	1	0
8-23	40	20	5	50	57	22	71	46	19
9-5	3	25	37	1	18	106	10	50	103
9-15	1	5	58	1	6	116	8	20	132
TOTAL	50	51	100	92	83	244	124	117	254